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Small bioreactor platform capsules provide persistent digestive biomass for continuous bioreactors operated under short hydraulic retention times

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ABSTRACT

Bioaugmentation treatments in general and the use of encapsulated selected microbial cultures in particular are gaining significant attention as a promising approach for the treatment of wastewater containing phenols and other organic pollutants. Thus, the purpose of this study is to investigate the biodegradation performance of a *Pseudomonas putida* culture encapsulated in a unique 3-D capsule known as the small bioreactor platform (SBP). Batch and continuous bioreactors, bioaugmented with two different *P. putida* culture states, i.e., encapsulated and free suspended cells, were operated under different phenol loadings. During the batch experiments, the biodegradation rate of the suspended culture was equal to or higher than the encapsulated culture, except for the highest phenol concentration of 1000 mg/L, where the encapsulated bacterial culture exhibited a superior biodegradation rate (45 mg/L/h) relative to the free culture (16.7 mg/L/h). In addition, in the continuous bioreactor, at a hydraulic retention time (HRT) of up to 1 h, the encapsulated *P. putida* completely biodegraded the phenol in the influent with 50 mg/L phenol, showing a maximal biodegradation rate of 45 mg/L/h. However, decreasing the HRT to 0.5 and 0.25 h resulted in incomplete biodegradation, despite higher biodegradation rates (90 and 70 mg/L/h, respectively). Furthermore, a sharp decrease in the biodegradation process efficiency was observed when the suspended culture was released from the SBP capsules by cutting the microfiltration membrane of the capsules. Therefore, this study demonstrates that, compared with suspended cultures, SBP culture encapsulation achieves superior performance as a bioaugmentation strategy, especially in continuous bioreactors, such that it can be applied in the treatment of phenol rich wastewater.

1. Introduction

Due to the presence of biological inhibitory substances in sewer systems throughout the year, domestic wastewater treatment plants (DWWTPs) can experience bio-process stress episodes, which can be characterized using the intensity of the inhibitory effect, as well as the duration of the stress episode (i.e., from its initiation until bioprocess recovery). During these stress episodes, waste biodegradability may decrease significantly, resulting in the production of low-quality effluent. Phenolic substances are important industrial chemicals that are widely used in wood preservation, petroleum refineries and petrochemical plants, coke gasifiers, paper and pulp manufacturing, pharmaceutical plants, food industries, plastics manufacturing, and agricultural activities. Their presence in DWWTP inflow is likely and can result in the inhibition of bio-processes and, in extreme cases, induce bio-process failure [1,2].

When phenols, which are aromatic organic compounds, are present in water or soil, they are considered problematic due to their toxic effects [3], such that studies have been conducted to characterize and optimize their biodegradation using phenol-rich wastewater. For large-scale phenol removal, previous studies have preferably employed biological treatment approaches, which are considered practical, environmentally friendly, and cost effective relative to chemical and physical treatments methods [4].

Based on these considerations, the bioaugmentation method is characterized by significant potential for the treatment of DWWTP containing phenolic compounds, i.e., supplementing selective bacterial cultures that show specific phenol-degradation capabilities in DWWTP biological processes should improve the treatment yield and process stability. However, for several reasons, including negative interactions between the bioaugmented culture and natural local micro-flora (e.g., protozoan predators), as well as the continuous dilution of the

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introduced bacterial culture in a continuous treatment system, achieving successful bioaugmentation treatment remains challenging [5–7].

To overcome the wash out and dilution of the bioaugmented culture, previous studies have developed and explored bacterial immobilization methods. The advantages and disadvantages of bioaugmented culture immobilization via suspension over the continuous treatment system have been described in several studies. The main advantages include high microorganism concentration retention in the reactors; availability of physical protection that increases the tolerance of the cells to toxic substances and environmental conditions, such as temperature, pH, and toxic substances, while maintaining a high culture density; and viability in a specific location in the water treatment process [7–9].

The culture immobilization/encapsulation procedure involves the use of different materials/matrices. For example, calcium alginate, starch and cellulose, activated carbon, and pumice are natural materials made via a relatively simple process and can be used to immobilize microbial cells. On the other hand, polyvinyl alcohol (PVA), sol-gel, and nanofibers are synthetic polymers produced via a more complicated process and may be used in addition to matrices for living cells [10–12]. However, several limitations are still associated with the use of such culture immobilization technologies. In most cases, the bacterial culture is encapsulated within a solid or semisolid formulation, leading to a slower contaminant biodegradation rate due to a decrease in the rate of diffusion through the solid or semisolid matrix. The resulting slower contaminant removal rate may not be synchronized with the DWWTP bioreactor hydraulic retention time (HRT), such that it may not be a practical solution nor cost-effective for DWWTPs. Overcoming this setback and realizing an efficient bioaugmentation practice requires a greater amount of the immobilized biomass. In addition, encapsulation using gels, such as alginate and others, may result in particle structure instability in aggressive environments (i.e., shear forces), resulting in the slow trafficking of oxygen and CO_2 to the cells. In addition, there is limited growth due to a lack of physical space within the particle. All of these disadvantages associated with immobilization techniques prevent the actual use of the encapsulation technique in DWWTPs.

According to El Fantroussi and Agathos [13], the most effective bioaugmentation implementation approach involves the use of a confined environment. A confined aquatic medium can support the introduced external bacterial culture, thus elevating the probability for adaptation and growth. The small bioreactor platform (SBP) technology provides a practical solution that can be employed to overcome the challenges associated with encapsulation, given that it creates a physical barrier between the introduced culture and activated sludge flora in the bioreactor. In addition, the SBP offers the possibility of overcoming biomass loss due to dilution and washout while excluding the control culture implementation site within the host bioreactor. The introduced bacterial culture grows inside the suspended SBP capsule, thus allowing the preservation of the natural bacteria physiology state. The results of our previous study show that the use of SBP capsules resulted in rapid phenol biodegradation rates that were similar to those resulting from the use of suspended cultures in batch treatment models, indicating that the SBP technology has potential for application in DWWTP batch bioreactors [9,14].

The SBP technology allows for the introduction of an exogenous bacterial culture into a wastewater treatment bioreactor under conditions that favor the preservation of high concentrations of the introduced bacterial culture in a suspended state within the SBP capsules for several weeks (> 8 weeks). Based on existing literature, all previous studies involving the use of the SBP technology have been related to the batch treatment process [2,5,9,14]. Therefore, the novelty of this study is the use of the SBP culture encapsulation technology in a continuous phenol biodegradation model using a *Pseudomonas putida* culture. In other words, in this study, we investigate the performance of SBP encapsulated *P. putida* cultures in the biodegradation of phenol in continuous and batch treatment models.

To examine the efficiency and advantages of the encapsulated bacteria culture as a bioaugmentation strategy in continuous bioreactors, we designed a continuous bioreactor operation process and operated it as a fluidized bed bioreactor, in which the SBP capsules flow freely in the medium. To investigate whether the SBP encapsulation procedure actually prevents bacteria wash out during the operation of the continuous bioreactors while maintaining sufficient biomass for the induction of a selective catabolic path under considerable DWWTP HRTs, we operated the bioreactor at short HRT rates.

2. Materials and methods

2.1. Encapsulated culture and activation procedure

A strain of *P. putida* F1 (ATCC 700007) was purchased and provided by Prof. Carlos Dosoretz from the Technion, Israel Institute of Technology. The culture was encapsulated with SBP capsules as previously described for other microbial species [5]. The capsules were made from a spheroidal cellulose acetate microfiltration membrane (pore size ranging from 0.2–0.7 μm), which served as a physical barrier between the encapsulated *P. putida* culture and the outer bioreactor medium. The structure, composition, and validation of the SBP capsule technology are presented in detail in the patent article [15] (Fig. 1).

Fig. 1 shows the SBP capsule that was used in this study. This capsule contains microbial cultures that were occluded in a confined environment with the help of a 3-D microfiltration membrane. The capsule physically separated the encapsulated microbial culture medium from the outer natural water microbial flora while allowing the diffusion of dissolved molecules, such as nutrients, organics, and gases, via its membrane. The advantage of the biotechnological SBP encapsulation method is that it provides a unique microenvironment for the selected culture to perform the desired biological purification process while ensuring that the introduced culture is protected within the bioreactor medium. Additionally, this protective membrane reduces a portion of the natural selective forces that may harm the introduced

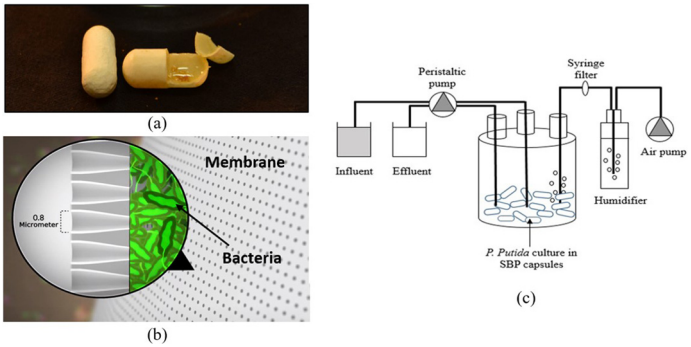

Fig. 1. Illustrations of a bioreactor bioaugmented with a *P. putida* culture encapsulated within small bioreactor platform (SBP) capsules. (a) An intact/cut SBP capsule. (b) Schematic illustration of the SBP capsule's microfiltration membrane. (c) Schematic diagram of a continuous bioreactor treatment process using the SBP technology to treat phenol.

culture (e.g., predation and other natural interactions among microorganisms).

To acclimate the inner culture to the phenol containing media, all *P. putida* containing SBP capsules were submerged in a 1000 mL flask filled with 300 mL of a sterile minimal salt medium (MSM) supplemented with 50 mg/L of phenol, after which the flask was stirred using an orbital shaker at 100 rpm at a constant temperature of 36 °C. The bacterial culture was then grown for five days.

2.2. Experimental setup

2.2.1. Batch bioreactor setup

The batch bioreactor experiment was primarily used to characterize the phenol-biodegradation rate of the SBP encapsulated *P. putida* culture. To further understand the effect of the microfiltration membrane barrier, phenol-biodegradation rate experiments using a suspended bacterial culture (*P. putida*) from cut capsules were also performed.

The phenol containing synthetic wastewater was prepared as previously described [9]. Treatments were conducted with the following initial phenol concentrations: 25, 50, 100, 500, and 1000 mg/L, where each solution to be treated was prepared in 250 mL Erlenmeyer flasks by adding phenol to 100 mL of MSM. For the intact/encapsulated treatment, one intact SBP capsule was transferred into an Erlenmeyer flask. In contrast, for the cut capsule experiments, one capsule was cut using a sterile scissor and the content of the capsule was poured into 100 mL of phenolic medium together with the capsule's shell. To monitor the variation of phenol concentration without any biodegradation throughout the experimental period, control experiments were performed. In the control flask containing the same MSM media and phenol concentrations, no bacterial seed was added. Finally, at a temperature of 36 °C and using an orbital shaker (100 rpm) as an agitator, all treatment and control experiments were performed in triplicate. The batch bioreactor experiments involved the following treatments: Erlenmeyer flasks with MSM supplemented with five different initial phenol concentrations, each containing one intact capsule; Erlenmeyer flasks with MSM supplemented with five different phenol concentrations, each containing one cut capsule; and Erlenmeyer flasks containing sterile controls, without the capsule. To determine the phenol concentration, samples were collected under sterile conditions at different time points.

Batch experiments were performed to explore the phenol biodegradation rate due to the action of the occluded biomass inside the microfiltration membrane confined environment in comparison with suspended cultures. The suspended and encapsulated bacterial cultures were harvested from the same bacterial source reservoir, i.e., the SBP capsules. While the lag phase was calculated from the start of the experiment until a sharp decline in phenol concentration for each treatment, the biodegradation rate was calculated based on the decline in the phenol concentration between the end of the lag phase and the point at which there was full removal of the phenol from the solution.

2.2.2. Continuous bioreactor setup and operation

The continuous bioreactor was operated as a fluidized bed bioreactor, in which the bed was actually the SBP capsules encapsulating the *P. putida* culture. Using water recirculation through a water jacket (Julabo, Seelbach, Germany), the temperature of the bioreactor was maintained at 30 °C. To ensure an adequate supply of oxygen to the culture, sterile air was bubbled through a diffuser located at the bottom of the reactor. To prevent evaporation of the bioreactor medium, the air was allowed to flow through a Millipore (Massachusetts, U.S.A.) syringe filter (0.22 μm) and a humidifier before entering the bioreactor. To sample the reactor influents and effluents, a two-channel head peristaltic pump (Masterflex, Cole-Parmer, Illinois, U.S.A.) was sent into the bioreactor through the bioreactor head plughole. The entire experimental setup and media were autoclaved for 17 min at 121 °C.

To avoid the spontaneous and incidental decrease in the concentration of phenol, as well as to ensure that the decrease in its concentration was only a consequence of the biodegradation process, the reactor was operated under sterile conditions, i.e., without the SBP capsules. This experiment, which served as the control interval, was conducted using MSM supplemented with 50 mg/L of phenol at an HRT of 7 h (i.e., an influent flow rate of 21 mL/h). The constant MSM volume in the bioreactor was 150 mL. For analysis, samples were collected periodically during the experimental interval.

After analyzing the effluent from the control system and ensuring that there were no differences in the influent and effluent phenol concentrations (50 mg/L), 15 SBP capsules encasing the pure *P. putida* culture were inserted into the bioreactor medium for the phenol biodegradation rate experiments. To maximize the phenol removal efficiency in the continuous bioreactor, the flow rate was gradually increased to 21, 48, 78, 160, 320, and then 640 mL/h, resulting in HRT reductions of 7.0, 3.5, 2.0, 1.0, 0.5, and 0.25 h, respectively. To determine the phenol concentrations, samples were collected at 0.0, 0.5, and 1 h, and thereafter, every 2 h. When the effluent phenol concentration eventually decreased to 0 mg/L, a higher flow rate was employed for the next stage of the experiment while reducing the HRT. Fig. 1(c) illustrates the continuous treatment model.

During the experiments performed under the lowestHRT (0.25 h), as well as to characterize the biodegradation rate associated with the encapsulated culture when it becomes suspended, the capsules were cut using sterile scissors, transferring the culture into the bioreactor media and creating a homogenous planktonic culture in the entire bioreactor liquid volume.

2.3. Phenol concentration and biodegradation rate measurements

To determine the concentration of phenol in the media, a standard direct colorimetric procedure using 4-aminoantipyrin was performed at 500 nm (GENESYS 20, Thermo Fisher Scientific, Massachusetts, U.S.A.), as described in the Standard Methods for the Examination of Water and Wastewater [16].

The bacterial count and biodegradation rate calculations were performed as previously described [17]. For the batch bioreactor experiments, phenol biodegradation rates were determined from the logarithmic slope of the phenol concentration (mg/L) with respect to the time curve, where $phenol_{50}$ represents the time required for a 50 % reduction in the initial phenol concentration [2]. For the continuous bioreactor experiments, the phenol biodegradation rate, R (mg/L/h), was calculated as follows [18]:

$$R = \frac{(Si - Se)}{D} \quad (1)$$

where Si and Se represent the influent and effluent phenol concentrations (mg/L), respectively, and D represents the HRT (h).

3. Results and discussion

3.1. Phenol biodegradation via encapsulated and free P. Putida cells in a batch bioreactor

The aim of the batch experiments was to compare the phenol biodegradation performance and kinetics of SBP encapsulated and suspended *P. putida* cultures in a batch bioreactor process using several initial phenol concentrations. The results presented in Fig. 2 show that complete phenol biodegradation was achieved for all phenol concentrations. As shown in Fig. 2(a), there was no difference between the phenol biodegradation performance of the encapsulated and suspended cultures following the treatment of a solution with an initial phenol concentration of 25 mg/L. At higher phenol concentrations, however, differences in the performance of the two culture states were observed. Biodegradation rates increased proportionally with increasing initial phenol concentrations from 5.7 mg/L/h following the treatment of the

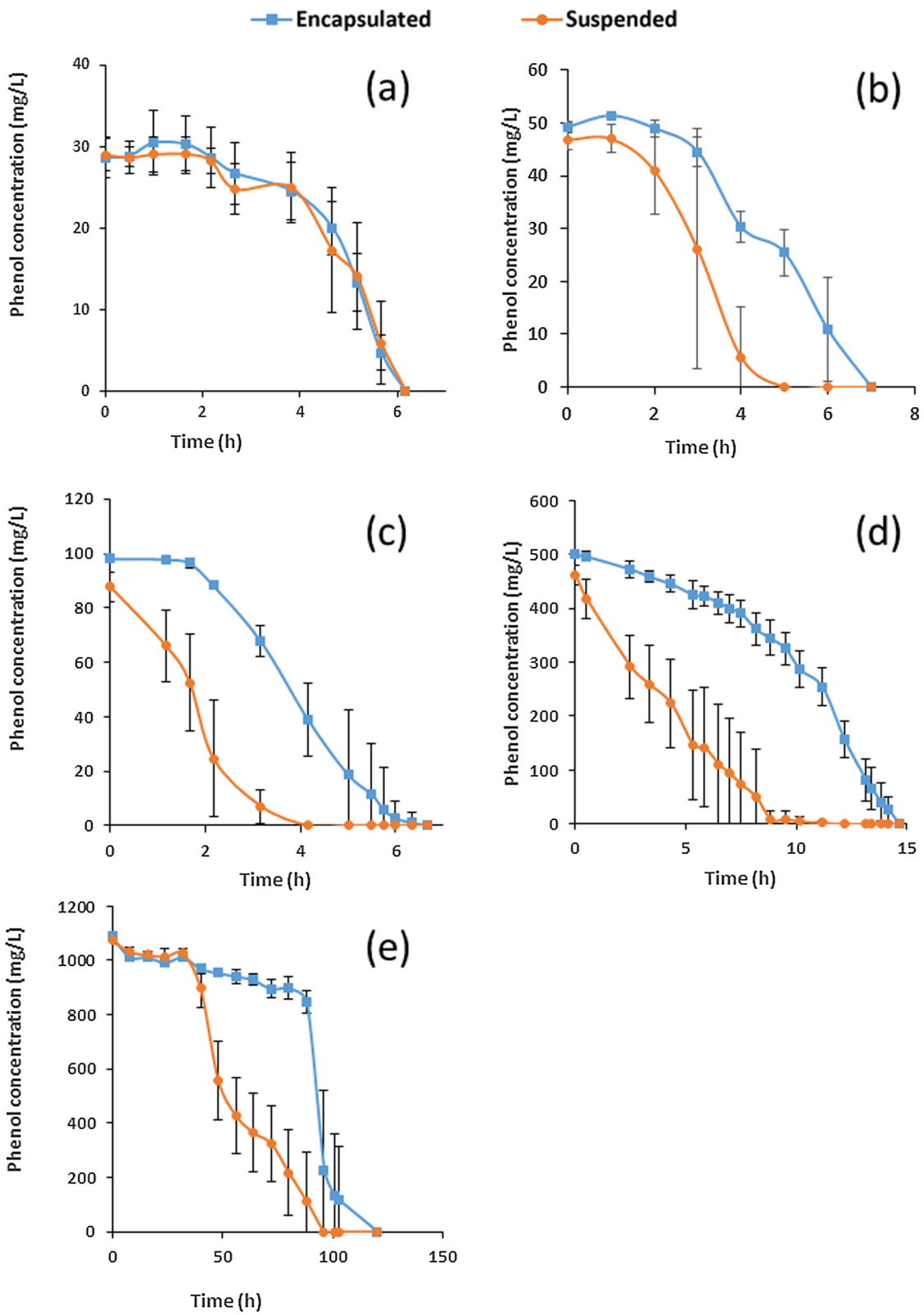

Fig. 2. Phenol biodegradation via the encapsulated and suspended *P. putida* cells in the batch bioreactor for several initial phenol concentrations. (**a**) 25, (**b**) 50, (**c**) 100, (**d**) 500, and (**e**) 1000 mg/L.

25 mg/L phenol solution to 60–62 mg/L/h following the treatment of the 500 mg/L phenol solution for both *P. putida* culture states (Table 1). While treating the 1000 mg/L phenol solution (Fig. 2(e)), the suspended culture showed a lower biodegradation rate (16.7 mg/L/h) relative to the encapsulated culture (45 mg/L/h). These results demonstrate that encapsulating the introduced culture under high phenol concentrations resulted in superior biodegradation rates, indicating that the 3-D confined environment provides a permeable confined protected environment.

The sterile controls did not show any change in the phenol

Table 1
Summary of the batch bioreactor experimental results showing a comparison of the action of the small bioreactor platform (SBP) for the encapsulated and suspension phenol-degrading bacteria at different initial phenol concentrations.

Phenol concentration (mg/L)	Suspension lag phase (h)	Capsule lag phase (h)	Suspension biodegradation rate (mg/L/h)	Capsule biodegradation rate (mg/L/h)	$Phenol_{50}$ suspension (h)	$Phenol_{50}$ capsule (h)	$Phenol_{50}$ suspension /$phenol_{50}$ capsule
25	2	2	5.7	5.7	4.5	4.5	1
50	1	2	16.7	13.3	2.5	4.5	0.55
100	0	2	35	26.7	2	4	0.5
500	0	8	62.5	60	3	13	0.23
1000	40	100	16.7	45	50	100	0.5

concentration due to different abiotic conditions (sorption, evaporation, etc.) (data not shown). Our previous study examined the adsorption and absorption of phenol to the microfiltration membrane of the SBP capsule. We showed that no significant adsorption and absorption exists and that the removal of phenol from the solution is only a biological process [9].

Bakhshi et al. [19] obtain similar observations in their study, in which batch bioreactor experiments were performed using a suspended *P. putida* culture. After 140 and 170 h, complete biodegradation was observed following the treatment of 300 and 500 mg/L phenol solutions, respectively. However, at higher phenol concentrations (700 and 1000 mg/L), the biodegradation efficiency decreased and was incomplete even after 170 h. In addition, Kumar and Kumar [20] also investigate phenol biodegradation using free *P. putida* cultures. They report complete phenol degradation after treating a 1000 mg/L phenol solution for 160 h. In our study, a higher biodegradation rate was observed after treatment for approximately 100 h.

Previous studies have reported that the cell number, bacterial strain, medium composition, environmental factors, and substrate concentration all affect the biodegradation rate [18]. In this study, given that the initial bacterial concentration in each Erlenmeyer flask resulted from a single SBP capsule (cut or intact), the average total encapsulated and suspended cell numbers in the 100 mL of media at the end of the experiments were 1.34×10^{10} and 3.0×10^{11} CFU/100 mL for the intact and cut capsule treatment, respectively. The higher bacterial numbers in the suspended treatment may be due to the lower bacterial population density in the medium compared with the SBP capsuled culture (inner volume of 2 mL), a condition which supports cell growth. Together with the diffusion through the capsule's membrane, this may explain the relatively lower biodegradation rate associated with the encapsulated culture (0–24 % lower) at the lower initial phenol concentrations.

During the batch experiments, the biodegradation rate of the suspended culture was equal to or higher than the encapsulated culture, except for the highest phenol concentration of 1000 mg/L, where the encapsulated bacterial culture showed a superior biodegradation rate. Excluding the 25 mg/L phenol solution, the encapsulated cultures showed a higher lag phase period for most of the initial concentrations, resulting in lower biodegradation rates, except for the solution with the highest initial phenol concentration (1000 mg/L), which showed a longer and more than 2-fold lag phase relative to the suspended culture. However, at this concentration, the encapsulated culture showed a higher biodegradation rate (45 mg/L/h) relative to the free cell culture (16.7 mg/L/h) (Table 1).

The 1000 mg/l treatment shows that *P. putida* is tolerant to high phenol concentrations as most bacteria will exhibit destructive effects to their membrane integrity in concentrations higher than 1000 mg/L [21,22]. Our results show that the encapsulated culture exhibits high biodegradation capabilities as compared with other reported isolates. For example, Yoneda et al. [23] show that *Rhodococcus opacus* PD630 exhibited increased phenol consumption rates at 20 mg/l/h after a long adaptation period. Yang et al. [24] obtain similar rates, as compared with our study, who describe the use of nanoparticles with *P. putida* biofilm that removed 62.5 mg/l/h of phenol with an initial phenol concentration of 500 mg/l after 8 h while the bacterial suspension showed a significantly slower biodegradation rate (35 mg/l/h). Shahryari et al. [21] examine a new *Acinetobacter* Sp. SA01, which was presented as an efficient phenol degrader bacterium. However, the isolate degrades 1000 mg/l in 60 h, which yields a rate of only 16.6 mg/l/h. In addition, after examining the phenol biodegradation performance of *P. putida* immobilized in alginate, Gonzalez et al. [25] report that the cultures at an initial phenol concentration of 1000 mg/L, after two adaptations, achieved full degradation in 250 h, with a lag phase of 100 h. In this study, for a similar phenol concentration, we observe a more rapid biodegradation rate (the cultures achieved full degradation after 120 h) and a similar lag phase duration.

$Phenol_{50}$ (P_{50}), which represents the time required for the initial phenol concentration to decrease by half, was calculated, followed by the determination of the P_{50} ratios of the encapsulated versus suspended culture treatments ($P_{50\ suspension}/P_{50\ SBP\ capsule}$) (Table 1). This ratio showed an increasing trend, from 1 up to 4.3, after which it decreased sharply to 2 at initial phenol concentrations of 25, 500, and 1000 mg/L, respectively, demonstrating the increasing difference in the ability of the encapsulated and suspended cultures to biodegrade phenol in solutions with increasing initial phenol concentrations. This finding is consistent with the results of our previous study [9], in which phenol biodegradation using suspended and encapsulated bacteria cultures were compared, yielding a P_{50} ratio (suspended versus encapsulated bacteria) of 0.5. Based on the results of previous studies and those of this study, we suggest that the initial phenol concentration plays an important role in the biodegradation process, even though at high phenol concentrations the culture state in the medium (suspended or encapsulated) is critical for the success of the process. The membrane of the SBP capsule provides a protective physical barrier against the outer environment, leading to a higher culture tolerance to high phenol concentrations, despite the longer lag phase. Nevertheless, for the implementation of this technology in real wastewater treatment reactors, the lag phase will only likely be observed at the beginning. As the estimated lifespan of the SBP capsule is 12 weeks, we expect that, after a few days of incubation, the biodegradation rate will become stable, such that the slower biodegradation effect from the lag phase will only be a temporary inhibition.

3.2. Phenol biodegradation via encapsulated P. Putida in a continuous bioreactor

The continuous bioreactor experiment was performed to estimate the maximal phenol removal capabilities of the SBP encapsulated *P. putida* cells. Initially, the bioreactor was operated as a sterile control (without any capsules), with an initial phenol concentration of 50 mg/L within the feed medium. The sterile control trial showed no phenol reduction, i.e., the phenol concentrations in the influent and effluent were identical following a 24 h operation of the bioreactor. Thereafter, the SBP encapsulated *P. putida* culture was introduced into the bioreactor medium. Fig. 3 presents a summary of the results of the continuous bioreactor experiment. At an HRT of 7.14, 3.57, 2, 1.29, and 1

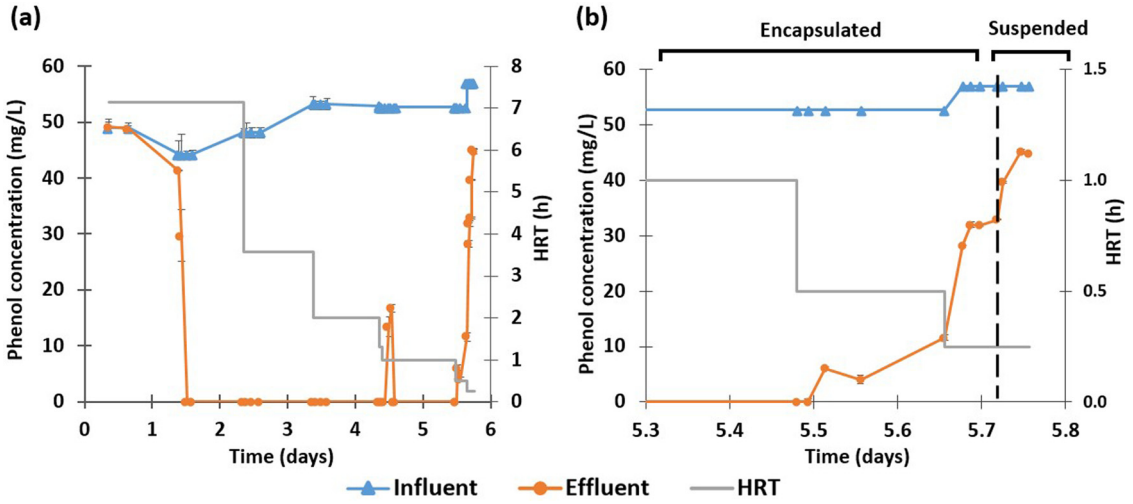

Fig. 3. (a) Influent and effluent phenol concentrations under different HRTs in the continuous bioreactor experiment (triangles and circles represent influent and effluent phenol concentrations, respectively, and the grey line represents the HRT value (h)). (b) Magnification (high resolution) of the results at day 5 when the SBP capsules were cut, at which point the encapsulated culture immediately became suspended in the bioreactor medium.

h, complete phenol biodegradation was observed, given that the phenol concentration in the effluents was 0 mg/L. We observed incomplete biodegradation only at a lower HRT of 0.5 h, where the residual phenol concentration within the effluents was 10 mg/L due to the short HRT. Decreasing the HRT to 0.25 h resulted in an increase in the residual phenol concentration in the effluent (up to 30 mg/L).

At this stage, we performed an interesting procedure. Unlike other encapsulation and immobilization methods, the SBP capsule technology offers the possibility of extracting suspended biomass from the inner part of the SBP capsule via a simple step, i.e., cutting the capsule using sterile scissors, such that, within one second, all encapsulated bacteria became freely suspended in the experimental medium. Thus, at an HRT of 0.25 h, the SBP capsules were cut, after which the encapsulated culture became suspended in the bioreactor medium. An analysis of the resulting effluent revealed an overall decrease in the efficiency of the biodegradation process, as well as the rapid accumulation of phenol (up to 45 mg/L) in the effluents. The result of this unique procedure serves as a proof of concept regarding the advantages of the SBP encapsulation method, which offers the possibility of overcoming the dilution of the introduced culture in bioaugmentation strategies due to a short HRT.

At the beginning of the continuous experiment at an HRT of 1 h, there was an accumulation of phenol in the effluent within the first 100 min, after which a faster biodegradation rate was achieved, resulting in complete biodegradation. We assume that an increase in phenol loading necessitated an adaptation period for the biodegrading cells.

Similar pattern changes resulting from changes in the HRT were observed in a previous study, in which a continuous bioreactor was operated with immobilized bacteria in alginate and polyurethane to remove dyes and toxicants from discharge wastewater [26]. In this previous study, the bioreactor was operated with increasing toxicant concentrations and flow rates. At all concentrations, including 1000 mg/L, complete biodegradation was observed when the flow rate was low. However, at higher flow rates, e.g., an HRT of 6 h, there was a substantial decrease in the biodegradation efficiency. Nevertheless, this study reveals that complete phenol degradation using a 50 mg/L phenol solution requires a minimum HRT of 1 h. At a lower HRT, residual phenol may persist in the effluent.

Gonzalez et al. [25] operate a continuous reactor experiment using suspended and immobilized (inside alginate beads) P. putida (P. putida (ATCC 17484) in an influent containing 1000 mg/L phenol. After gradually decreasing the HRT from 4 to 0.2 days, they found that the system was stable, where the immobilized culture was capable of completely degrading the phenol at a lower HRT (0.25 d), as compared with the suspended culture (0.3 d). They suggest that this difference resulted from a washed out suspended culture (culture dilution) due to high dilution rates. The SBP system can possibly present a biodigestion time that is significantly lower than those displayed by other encapsulation methods (HRT of 1 versus 6 h for the suspended versus encapsulated cultures, respectively). This advantage is critical in planning SBP technology-based continuous treatment plants. In other words, SBP culture encapsulation allows for the design and establishment of smaller bioreactors that are up to 6 times more efficient relative to other encapsulation technologies. This results in significant reductions in the capital costs associated with the establishment of DWWTPs.

We further investigated the effect that the flow rate has on the phenol biodegradation rate. As shown in Fig. 4, in the continuous bioreactor, the phenol biodegradation rate increased rapidly with an decrease in the HRT, e.g., at an HRT of 0.5 h, the maximum biodegradation rate was 90 mg/L/h, which then decreased moderately to 75 mg/L/h. As mentioned previously, at an HRT of 0.25 h, the capsules were cut, releasing the encapsulated cells, which became suspended within the bioreactor medium. Due to the continuous flow of influents, the influents were washed out from the system, resulting in the continuous dilution of the residual suspended biomass. Therefore, cutting the capsules had a significant effect on culture persistence within the reactor, which resulted in a decrease in the reactor treatment efficiency. Once the capsules were cut, the biodegradation rate decreased sharply by 50 % to 27 mg/L/h. Mordocco et al. [18] operate a continuous bioreactor using immobilized *P. putida* in alginate beads, as well as free

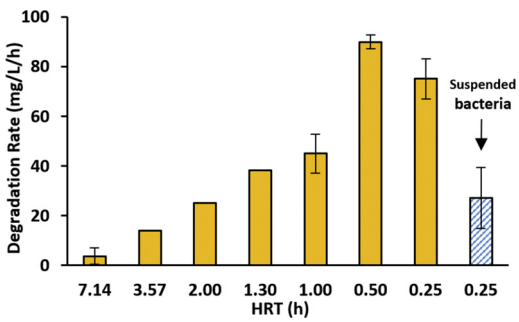

Fig. 4. Phenol biodegradation rates at different HRTs during the operation of the continuous bioreactor. Yellow columns represent encapsulated *P. putida* culture treatments, whereas the blue-lined column represents the result of the treatment with the cut SBP capsules.

cells in a reactor medium. Their results showed a higher biodegradation rate (108 mg/l/h) at longer HRTs 3.33–1.67 h), as compared with the results of this study. In their study, no changes in the biodegradation rate were observed at reduced HRT rates. However, in this study, we observed an increasingly higher biodegradation rate, i.e., up to an HRT of 0.5 h. Nevertheless, an important outcome of both studies is that the use of free *P. putida* cell cultures resulted in a decrease in the biodegradation efficiency compared with the immobilized culture state. Similarly, Ehrhardt and Rehm [27] investigate phenol biodegradation using *P. putida* P8 adsorbed onto activated carbon compared with free cells in a continuous flow system. Their study showed that the immobilized culture presented a higher biodegradation rate compared with the free cells. This higher degradation ability of the encapsulated cultures at high concentrations of the toxic substrate phenol also occurred in other studies, such as Juárez-Ramírez et al. [28], who examined a strain of C. tropicalis entrapped in agar beads that were able to degrade phenol at rates 10 times higher than the free state. Other polymers, such as PAAH [29] and polyacrylonitrile with glutaraldehyde as a coupling [30], exhibited better degradation rates for encapsulated cultures compared with free cultures.

We conclude that, at a short short HRT, as is the case with several bioaugmentation-based wastewater treatment processes, the immobilization and encapsulation of the microbial culture prevents the dilution and washout of the introduced culture.

Based on the existing literature, we can assume that encapsulation is a better technique for the immobilization process as in the majority of activated sludge bioaugmentation studies. Inoculation with a specific strain as a suspended biomass or even as a biofilm on bio-carriers generally has no effect or only a minimal contribution to the overall purification process. For example, in a study focusing on the benefits of bacterial immobilization in alginate beads as a protective barrier from predators, Covarrubias et al. [31] argue that, although the beads consisted of fine matter that facilitated immobilization, their outer layer was degradable, indicated that it may be degraded after operating for long periods of up to 48 h cycles. In contrast, in SBP capsules, the bacterial culture is suspended in a hollow durable microfiltration membrane, which essentially acts as a small bioreactor. El Fantroussi and Agathos [13] show that an effective bioaugmentation approach involves a confined environment rather than a simple supplementation of microbial biomass to the treated system. The SBP technology presents a practical solution for microbial cell entrapment that can be employed to overcome the challenges associated with other encapsulation techniques. In our previous study, we estimated the life span of the SBP capsules, whose results showed that, in the activated sludge wastewater treatment processes, the capsules can maintain a viable encapsulated culture for over three months [2].

4. Conclusions

The success of every bioaugmentation process depends on the effective implementation of the added culture into the host system and the persistence of the culture within the introduced environment (including the presence of toxic substances, such as phenols). This study demonstrates that, compared with suspended cultures, the SBP culture encapsulation technology can achieve an effective bioaugmentation process, especially in high phenol concentrations or sh under short HRTs in batch or continuous bioreactors, respectively. Therefore, the results of this study present a practical bioaugmentation method that can be used in phenol biodegradation processes under short HRTs (i.e., one hour or less). Future studies should focus on the biodegradation ability of the SBP encapsulated cultures in a fixed bed bioreactor under high flow rates, i.e., short HRTs. Moreover, an interesting setup may be to fill the entire column of a fixed bed bioreactor with different encapsulated bacterial species to biodegrade different organic pollutants in the treated water.

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Declaration of Competing Interest

The authors declare no conflicts of interest.

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